Appln. No.: 10/697,036

Docket No: Q78242

REMARKS

Upon entry of the Amendment, claims 1-14 are all the claims pending in the application. Claims 11-14 have been withdrawn. Claims 1, 3, 4, and 8 have been amended. The amendment to claim 1 is supported in the specification, such as on pages 59-60. The amendments to claims 3 and 4 have been made, in view of the amendment to claim 1. The amendment to claim 8 is supported in the specification, such as on page 45 and pages 46-47. Therefore, no new matter has been added.

I. Specification

The disclosure is objected to allegedly for including a hyperlink language.

Applicants have accordingly amended the disclosure.

II. Claim Rejections - 35 U.S.C. § 112

(A) Claims 1-10 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

Applicants respectfully traverse.

The Examiner asserts that the phrase "hybrid-sensor kinase" as recited in claim 1 is unclear. The Examiner asserts that the difference between a "hybrid-sensor kinase" and an osmosensing histidine kinase is unclear.

The specification describes that a hybrid-sensor kinase is involved in signal transduction pathway that involves three proteins. *See* page 13 of the specification. The specification describes that a hybrid-sensor kinase is composed of an input region, a histidine kinase region, and a receiver regions at the C-terminal end. *Id.* at 12. In the signal transduction pathway, a

Appln. No.: 10/697,036

Docket No: Q78242

phosphate may be transferred from a hybrid-sensor kinase (a sensor) to a response regulator via an intervening phosphotransmitter protein.

Further, the specification describes that an "osmosensing histidine kinase having no transmembrane region" refers to an osmosensing protein which has a repeat sequence region of amino acid sequences, a histidine kinase region and a receiver region, but no transmembrane region. See pages 26-27 of the specification. Each repeat is composed of about 90 amino acids

The specification describes the difference between the hybrid-sensor kinase and the osmosensing histidine kinase having no transmembrane region. See page 24 of the specification. In contrast to the input region of the hybrid-sensor kinase, the osmosensing histidine kinase having no transmembrane region. The input region of many hybrid-sensor kinases include a transmembrane region. See page 13. Instead of the transmembrane region, the osmosensing histidine kinase having no transmembrane region includes a repeat sequence region.

Claim 8 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being (B) indefinite.

The Examiner asserts that the phrase "derived from" is unclear.

Applicants have accordingly amended claim 8.

and shares amino acid sequence homology with the other repeats.

III. Claim Rejections - 35 U.S.C. § 102

Claims 1-3 and 5-10 are rejected under 35 U.S.C. § 102(b), as allegedly being anticipated by Cui et al. "An osmosensing histidine kinase mediates dicarboximide fungicide resistance in

10

Appln. No.: 10/697,036

Botryotinia fuckeliana (Botrytis cinerea)," Fungal Genetics and Biology, 36 (2002) 187-198 ("Cui").

Docket No: Q78242

Claim 1 presently recites that a cell is a bacterial cell, yeast cell, or a plant cell.

In contrast, Table 6 of Cui discloses B. fuckeliana resistant mutant strains. B. fuckeliana is not a bacterial cell, yeast cell, or a plant cell. B. fuckeliana is a filamentous fungi. See, e.g., pages 187-188 of Cui. In this regard, Cui fails to anticipate the transformed cell recited in claim 1.

Claims 2-3 and 5-10 depend directly or indirectly from claim 1. In this regard, Cui fails to anticipate claims 2-3 and 5-10 for at least the same reasons as claim 1.

IV. Claim Rejections - 35 U.S.C. § 103

Claim 4 is rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Cui.

Claim 4 indirectly depends from claim 1. Claim 1 presently recites that a cell is a bacterial cell, yeast cell, or a plant cell.

Referring to page 4 of the Office Action, it is the Examiner's position that a person of ordinary skill in the art would have been motivated to introduce Dafl into a S. cerevisae host cell either lacking a Sln gene or having a less active Sln gene. As described above, Cui is deficient in that it fails to describe or teach that the cell is bacterial cell, yeast cell, or plant cell. The Examiner asserts that Cui teaches that the histidine kinase pathway in B. fuckeliana and S. cerevisae are very similar pathways involving MAP kinases and transcription factors.

Cui proposes that Dafl encodes the first enzyme in an osmotic pathway in B. fuckeliana that operates in a similar manner to that in budding yeast. See, left column, page 195 and Figure

11

Appln. No.: 10/697,036

6. Cui teaches that *Bos1* gene of *B. fuckeliana* encodes a protein that exhibits characteristic HK features, including the conserved H-, X-, N-, D-, F-, and G-boxes. *See*, page 191, left column. Figure 3 of Cui shows that amino acid sequence homology between a protein encoded by the *Bos1* gene and a protein encoded by the *Sln1* gene is restricted to short conserved regions encompassing the phosphorylated histidine and receiver aspartic acid residues. Cui teaches that the protein encoded by *Bos1* gene of *B. fuckeliana* possesses six 90-amino acid repeat motifs near the N-terminus thereof. *See*, page 191, left column. Cui also teaches that the N-terminus shares homology with bacterial soluble sensory transducers and there is no transmembrane domain within the predicted protein, indicating that the protein is localized in the cytoplasm. *Id*. Cui teaches that wild-type *B. fuckeliana* is sensitive to antifungal compounds such as dicarboximide.

Docket No: Q78242

Applicants respectfully submit that a person of ordinary skill in the art would not have been motivated to introduce *Daf1* into a bacterial cell, yeast cell, or plant cell. As described in more detail below, the rejection amounts to an "obvious to try" standard, which is the incorrect standard for patentability. MPEP § 2145 (X)(B). Cui, at best, provides a general approach without giving any direction as to which of many possible choices is likely to be successful. Further, there is no reasonable expectation of success that the *B. fuckeliana* protein encoded by the *Daf1* gene would operate in an osmotic response pathway in *S. cerevicia*. MPEP § 2143.02.

The Sln1 gene from S. cerevisae encodes a histidine kinase having transmembrane regions near the N-terminus and having no repeat motifs. Wild-type S. cerevisae is also not sensitive to antifungal compounds. Further, the amino acid sequence homology in Figure 3 of

Appln. No.: 10/697,036

Docket No: Q78242

Cui is evidence that a person of ordinary skill in the art would not have been motivated to introduce the Dafl gene into a S. cerevisae host cell either lacking a Sln gene or having a less active Sln gene.

Applicants submit herewith a copy of Nagahashi, et al. "Isolation of CaSLN1 and CaNIK1, the genes for osmosensing histidine kinase homologues, from the pathogenic fungus Candida albicans," Microbiology (1998), 144, 425-432 ("Nagahashi").

Nagahashi is evidence that a person of ordinary skill in the art would not have reasonably expected the Dafl gene to operate in a S. cerevisae host cell either lacking a Sln gene or having a less active Sln gene. Nagahashi teaches producing SLN1 deficient S. cereviciae cells harboring a CaNIK1 gene in a multicopy plasmid. See page 430, right column. The protein encoded by the CaNIK1 gene ("CaNikp") is from Candida albicans. CaNikp (1) has regions that are related to the sensor kinase and response regulator domains of two-component histidine kinase systems, (2) contains five repeats of about 90 amino acids with the N-terminal half thereof, and (3) lacks any apparent transmembrane domain. See, page 427, right column to page 430, left column. Nagahashi teaches that the SLN1 deficient S. cerevisiae cell harboring the CaNIK1 gene cannot grow. See page 430, right column. In view of its inability to grow, Nagahashi proposes that CaNIK1 is functionally distinct from S. cerevisiae SLN1 and that CaNIK1 may not act in the same pathway thereof. Id. As S. cerevisiae and Candida albicans are both forms of yeasts. Nagahashi also teaches that there may be a divergence in osmosensing signal transduction mechanisms in yeasts. See page 431.

Appln. No.: 10/697,036

Docket No: Q78242

In this regard, similar to CaNIK1, a person of ordinary skill in the art would not have

reasonably expected Dafl to operate in a S. cerevisae host cell either lacking a Sln gene or

having a less active Sln gene. Cui does not provide teachings particular enough that would

motivate a person of ordinary skill in the art to select *Daf1* or that would provide a reasonable

expectation of success.

Thus, Claim 4 is not obvious at least by virtue of its dependence from claim 1.

In view of the above, reconsideration and allowance of this application are now believed

to be in order, and such actions are hereby solicited. If any points remain in issue which the

Examiner feels may be best resolved through a personal or telephone interview, the Examiner is

kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue

Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any

overpayments to said Deposit Account.

Respectfully submitted,

Registration No. 58,490

SUGHRUE MION, PLLC

Telephone: (202) 293-7060

Facsimile: (202) 293-7860

WASHINGTON OFFICE 23373

CUSTOMER NUMBER

Date: April 16, 2007

14

Isolation of CaSLN1 and CaNIK1, the genes for osmosensing histidine kinase homologues, from the pathogenic fungus Candida albicans

Shigehisa Nagahashi,^{1,2} Toshiyuki Mio,² Naomi Ono,² Toshiko Yamada-Okabe,³ Mikio Arisawa,² Howard Bussey¹ and Hisafumi Yamada-Okabe²

Author for correspondence: Hisafumi Yamada-Okabe, Tel: +81 467 47 2242. Fax: +81 467 46 5320. e-mail: hisafumi.okabe@roche.com

- Department of Biology, McGill University, 1205 Dr. Penfield, Montreal, Quebec, Canada H3A 1B1
- ² Department of Mycology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247, Japan
- ³ Department of Hygiene, School of Medicine, Yokohama City University, 3-9, Fukuura, Kanazawaku, Yokohama 236, Japan

Recent studies have revealed that fungi possess a mechanism similar to bacterial two-component systems to respond to extracellular changes in osmolarity. In Saccharomyces cerevisiae, SIn1p contains both histidine kinase and receiver (response regulator) domains and acts as an osmosensor protein that regulates the downstream HOG1 MAP kinase cascade. SLN1 of Cańdida albicans was functionally cloned using an 5. cerevisiae strain in which SLN1 expression was conditionally suppressed. Deletion analysis of the cloned gene demonstrated that the receiver domain of C. albicans Sln1p was not necessary to rescue SLN1-deficient S. cerevisiae strains. Unlike S. cerevisiae, a null mutation of C. albicans SLN1 was viable under regular and high osmotic conditions, but it caused a slight growth retardation at high osmolarity. Southern blotting with C. albicans SLN1 revealed the presence of related genes, one of which is highly homologous to the NIK1 gene of Neurospora crassa. Thus, C. albicans harbours both SLN1- and NIK1-type histidine kinases.

Keywords: Candida albicans, cloning, osmosensor, two-component system, histidine kinase

INTRODUCTION

Two-component systems which involve a phosphorelay from the histidine of the sensor kinase to the aspartic acid of the response regulator are widespread in bacteria (Bourret et al., 1991; Stock et al., 1991; Parkinson & Kofoid, 1992). Regulatory proteins similar to bacterial two-component systems are also found in some eukaryotes (Brown et al., 1993; Ota & Varshavsky, 1993; Alex et al., 1996; Chang et al., 1993; Hua et al., 1995; Wilkinson et al., 1995). In Saccharomyces cerevisiae, Sln1p consists of an extracellular sensor, a kinase and a receiver domain (Ota & Varshavsky, 1993; Maeda et al., 1994) and acts as an osmosensor protein (Maeda et al., 1994). Under low osmolarity conditions, a specific histidine in the kinase domain is autophosphorylated. The phosphate moiety of this histidine is first transferred to a certain aspartic

acid within the receiver domain and then via a phosphorelay to the downstream proteins Ypd1p and Ssk1p, leading to the shut off of the HOG1 MAP kinase cascade (Brewster et al., 1993; Maeda et al., 1994; Posas et al., 1996). Histidine kinase activity and phosphorylation of Sln1p are essential for growth at low osmolarity because a mutation of either the autophosphorylating histidine or the receiver aspartic acid of Sln1p is lethal under these conditions (Maeda et al., 1994). Increased osmolarity hampers the histidine kinase activity of Sln1p, which in turn activates downstream HOG1 MAP kinase (Brewster et al., 1993; Maeda et al., 1994; Posas et al., 1996).

Involvement of a histidine kinase in an osmosensing pathway has also been reported in *Neurospora crassa* (Alex et al., 1996). The predicted product of the N. crassa NIK1 gene possesses two domains that are related to sensor histidine kinases and response regulators of bacterial two-component proteins (Alex et al., 1996). Nik1p is an apparent cytoplasmic protein with six repeats of about 90 aa that may form a coiled-coil structure. Deletion of the NIK1 gene caused aberrant

Abbreviation: 5-FOA, 5-fluoroorotic acid.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are AB006362 (SLN1) and AB006363 (NIK1).

hyphal morphology, a phenotype more prominent under high osmotic conditions (Alex et al., 1996).

To address whether a histidine kinase osmosensing mechanism is conserved in other yeasts, we attempted the functional cloning of Candida albicans SLN1. In addition, probing a C. albicans genomic library with the C. albicans SLN1 gene identified a gene that is highly related to NIK1 of N. crassa. Thus, it seems that C. albicans has both Sln1p- and Nik1p-type histidine kinases which may allow adaptation to different osmotic conditions.

METHODS

Plasmid construction and yeast strain. A 700 bp HindIII-HindIII fragment that harbours the tetO-HOP1 chimaeric promoter, UAS and URS, was excised from p97t (Nagahashi et al., 1997), ligated with the DNA fragment containing the hisG-URA3-hisG module isolated from pMPY-ZAP (Schneider et al., 1996) and cloned into Bluescript SKII+ to generate p97tZAP. Replacement of the cognate SLN1 promoter with a tetracycline-controllable promoter (Nagahashi et al., 1997) was achieved by the one-step gene replacement method (Baudin et al., 1993; Schneider et al., 1996) with slight modification. DNA fragments harbouring target sequences of the SLN1 and tetracycline-regulated promoters and the hisG-URA3-hisG module were amplified by PCR using p97tZAP as a template and a pair of primers, 5' CATCGAAAACAGCACGAACAAAAGCCAACTCAC-TACATTTTAGAACAGCTATGACCATG 3' and 5' TCC-AATTTTGATGCCAGGCCAAATCGCATTTGTATT-GGAATTCTTTTCTGAGATAAAG 3'. The resulting DNA fragment was transfected into an S. cerevisiae strain, YPH499 (MATa ade2 his3 leu2 lys2 ura3) that had been transfected with pINFAGAL4 and which constitutively expressed the tetR-GAL4 fusion activator (Nagahashi et al., 1997). After confirming the correct integration of the tetO-HOP1 chimaeric promoter by PCR and Southern blotting, transfectants were selected by 5-fluoroorotic acid (5-FOA) and used for experiments. For convenience, we designated the above strain as Tet-SLN1. To determine the region of CaSLN1 essential for complementing ScSLN1, deletion mutants of CaSLN1 were cloned into the unique BamHI site of YEp24T (Yamada-Okabe et al., 1996) and transfected into another S. cerevisiae strain, A451 (MATa can1 leu2 trp1 ura3 aro7) in which the original SLN1 locus was disrupted by LEU2 but where episomal copies of SLN1 in pYES2 (Invitrogen) were maintained under the control of the GAL1 promoter. This strain, termed pYES-SLN1, was unable to grow in the presence of either 5-FOA or glucose as sole carbon source. Primers used to amplify ScSLN1 were 5' CCCGGGGAATTCATGCGAT-TTGGCCTGCCA 3' and 5' CCCGGGGAATTCTCATT-TGTTATTTTCTT 3'. For ScSLN1 disruption, the 2.3 kb PstI-PstI region of ScSLN1 was replaced by LEU2.

Screening the C. albicans SLN1 and NIK1 genes. Tet-SLN1 cells were transfected with a C. albicans genomic DNA library and spread on plates containing 50 µg tetracycline ml⁻¹. After incubation at 30 °C for 3 d, colonies appeared on the plates, cells were collected and plasmid DNA was recovered from them. After a second screening, the essential region of the insert DNA that conferred tetracycline-resistant growth of Tet-SLN1 was determined by cloning each DNA fragment in YEp24T (Yamada-Okabe et al., 1996) and in pRS416 (Stratagene).

For cloning the NIK1 gene of C. albicans, a C. albicans genomic DNA library was screened with the 2·1 kb KpnI-Sal1 fragment of the C. albicans SLN1 gene as probe. Hybridization was carried out under low stringency conditions in a buffer containing 0·25 M sodium phosphate (pH 7·2), 2×SSC (1×SSC contains 150 mM NaCl and 15 mM sodium citrate), 1% (w/v) bovine serum albumin, 1 mM EDTA, 0·1% (w/v) SDS and 25% (w/v) formamide at 37 °C for 12 h. Radiolabelling of DNA with $[\alpha^{-32}P]dCTP$ and DNA sequencing were carried out as described by Sambrook et al. (1989). Construction of C. albicans genomic DNA library was reported previously (Yamada-Okabe et al., 1996).

Disruption of CaSLN1. The 2.3 kb KpnI-KpnI fragment of CaSLN1 was cloned in pUC19 and the resulting plasmid digested with Ball and SnaBI followed by ligation with a 3.8 kb BamHI-XbaI fragment carrying the hisG-URA3-hisG module, generating pCASLN1U. Thus, the 0.6 kb SnaBI-Ball region of CaSLN1 was replaced by the hisG-URA3-hisG module in pCASLN1U. After pCASLN1U was linearized by digestion with PvuII, 10 µg DNA was transformed into C. albicans CAI4 (ura3\Delta::imm434/ura3\Delta::imm434) cells by the lithium acetate method (Sanglard et al., 1997). Before and after a second round of transformation, the URA3 gene was excised by 5-FOA as described previously (Mio et al., 1996). Unless otherwise specified, C. albicans cells were cultured in YPD (1%, w/v, yeast extract; 2%, w/v, peptone; 2%, w/v, glucose) in the presence or absence of 1.5 M NaCl.

RESULTS

Functional cloning of the C. albicans SLN1 gene

To isolate a *C. albicans SLN1* gene by functional cloning, we generated an *S. cerevisiae* strain in which *SLN1* expression was conditionally repressed. This strain, designated Tet-SLN1, grew normally in the absence of tetracycline, while its growth was severely impaired by the addition of 50 µg tetracycline ml⁻¹. Transfection of Tet-SLN1 with intact *S. cerevisiae SLN1* restored normal growth even in the presence of 50 µg tetracycline ml⁻¹, demonstrating that the growth defect of Tet-SLN1 caused by tetracycline was largely due to the repression of *SLN1* expression.

Tet-SLN1 cells were transfected with a C. albicans genomic DNA library that was constructed with a vector harbouring the S. cerevisiae TRP1 gene and a 2 µ replication origin (Yamada-Okabe et al., 1996) and transfectants were selected using 50 µg tetracycline ml⁻¹. From 10⁴ independent colonies, three clones grew in the presence of 50 µg tetracycline ml⁻¹. The plasmid DNA was recovered from these clones and a restriction map of each insert DNA determined. Although the restriction maps of these three clones differed from each other, the map of clone 1 coincided with the pattern of a C. albicans genomic Southern blot that was obtained using the S. cerevisiae SLN1 gene as a probe and this clone was analysed further.

Deletion analysis with this clone demonstrated that a 5.6 kb EcoRV-XhoI region was sufficient to rescue Tet-SLN1 cells in the presence of tetracycline (Fig. 1a). Sequencing of this region revealed that it contained a long ORF of 3.6 kb, with a coding sequence possibly

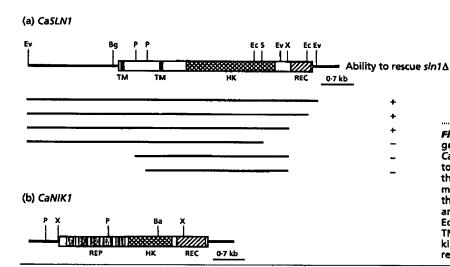


Fig. 1. Restriction maps of C. albicans genomic DNA fragments that contain CaSLN1 (a) and CaNIK1 (b) are illustrated together with the expected structures of their products. The ability of deletion mutants to rescue pYES-SLN1 cells (sIn1Δ) in the presence of 5-FOA is indicated as '+' and '-', respectively. Ba, BamHI; Bg, Bg/II; Ec, EcoRI; Ev, EcoRV; P, Psti; S, Safi; X, Xhoi; TM, transmembrane domain; HK, histidine kinase domain; REC, receiver domain; REP, repeats of an approximately 90 aa motif.

extending further downstream. To obtain a clone containing the missing 3' end of the ORF, we screened the same C. albicans genomic DNA library using a 0.5 kb Sall-XhoI fragment as a probe. Sequencing of a clone containing the complete ORF revealed that the predicted product of the gene is a 150 kDa protein highly similar to S. cerevisiae Sln1p (37 % identity) (Fig. 2a) and the gene was designated C. albicans SLN1 (CaSLN1). To avoid confusion, the S. cerevisiae SLN1 was termed ScSLN1 in this study. Like ScSln1p, CaSln1p has no Nterminal signal sequence, but possesses two potential transmembrane helices in its N-terminal half (Fig. 3a). Sequence identity between CaSln1p and ScSln1p was remarkably high within the histidine kinase and receiver domains located in the middle of the protein and near the C-terminal end, respectively, and both the essential phosphorylating histidine at position 576 and receiver aspartic acid at position 1144 of ScSln1p are conserved in CaSln1p (Fig. 2a).

As described above, the initially isolated CaSLN1 clone lacked the C-terminal receiver domain, suggesting that the receiver domain of CaSln1p is not crucial to complement a ScSLN1 deletion. We confirmed this by making a series of deletion mutants and transfecting them into another S. cerevisiae strain, pYES-SLN1. Consistent with the results of the functional cloning of CaSLN1, the 5.6 kb EcoRV-XhoI fragment of CaSln1p still rescued pYES-SLN1 in the presence of 5-FOA or glucose, while further deletion from C terminus, destroying half of the probable ATP-binding site within the histidine kinase domain lead to loss of the ability to complement ScSLN1 (Figs 1a and 4).

Disruption of CaSLN1

To study the function of CaSLN1, we generated the homozygous casln1 Δ null mutant strain by means of the ura-blaster protocol (Fonzi & Irwin, 1993). Using this

strategy, the first one-third of the histidine kinase domain, including the probable autophosphorylating histidine at position 519 of CaSln1p, was replaced by the hisG sequence of Salmonella typhimurium (Fig. 5a). The correct integration of the hisG sequence into the expected loci was confirmed by Southern blotting (Fig. 5b). Unlike S. cerevisiae, the homozygous casln1\Delta null mutant of C. albicans grew under both normal and high osmotic conditions and sustained an ability to form hyphae. However, the homozygous null mutation, but not the hemizygous mutation of CaSLN1 caused weak growth retardation in the presence of 1.5 M NaCl (Fig. 6a). Moreover, the homozygous casin1∆ null mutant cells elongated in the presence of 1.5 M NaCl (Fig. 6b). Similar morphological changes of the homozygous casln14 null mutants were also observed in the presence of 1 M sorbitol or 1 M KCl (data not shown).

The above results clearly demonstrate that CaSLN1 is not an essential gene in C. albicans and may imply that C. albicans harbours other genes, possibly histidine kinases, to adapt to high osmolarity.

Cloning the C. albicans NIK1 gene

In an attempt to search for other histidine kinase genes in C. albicans, we performed genomic Southern blotting with a part of CaSLN1 DNA corresponding to the histidine kinase domain of CaSln1p and detected several discrete bands that were not derived from CaSLN1 alleles. A genomic DNA library was again screened with the 2·1 kb KpnI-EcoRI fragment of CaSLN1 as a probe and a clone distinct from CaSLN1 was obtained. This clone contained an ORF that could encode a 119 kDa protein highly similar to N. crassa Nik1p (50% identity) (Fig. 2b) and the gene was termed C. albicans NIK1 (CaNIK1). Like N. crassa Nik1p, CaNik1p contains five repeats of about 90 as within the N-terminal half (Figs 1b and 2b) and a hydropathy plot of CaNik1p lacks any apparent transmembrane domain (Fig. 3b). However,

| (a) | | | | | | | | |
|----------------|-----------------------|----------------------------|---------------------------|--------------------|---------------------|-------------|------------|-------------|
| ScSln1p 1 : | MRFGLPSKLE LTPPFRIGI | | | | | | | SSLTSYVAGN |
| CaSlnlp 1 : | MRRLRIGII | | VIGIYESANL | | ISQLKRTQVQ | ONICATEOR | MIVSEVDSLT | VPLSNYRACIN |
| ScSlnlp 101 : | KSADNWVDSL SVIQKFLSS | NLFYVAKVYD SSFNAVLNAT | NNGTGDLIPE | | TOTPLPSSLE | | | |
| CaSlnlp 90: | nskavpseaq nylqqyvlit | DSFTAARLYD LDLOVVASSE | | | | | | RYFNGITVPV |
| ScSlnlp 194 : | FANPSIILTD SRVYGYITI | MSABGLKSVP NOTTALEHST | LAIISAVY | NSQ | GKASGYHFV- | -FPPYGSRSD | LPQKVFSIKN | DIFISSAFRN |
| CaSlnlp 188 : | LENSSIILSQ PSISGYLTI | / AAAESIRSAL NSTSEDDYQA | MA-VQPVYGD | | | | SLLEAGTIYN | INSSSSMETA |
| ScSlnlp 283 : | GKGGSLKQTN ILSTRIVE | A LGYSPCSF NLVNWVAIVS | QPESVFLSPA | TKLAK <u>IITGT</u> | VIAIGVFVIL | LTLPLAKWAY | QPIVRLQKAT | ELITEGRGLR |
| CaSln1n 287 : | LASNSGTATG VKSFFGKKV | TGPSRISUON NINASTUTU | * *** ** * OSNEVENCEDA | | | * *** * | | |
| | | | | | | | | |
| ScSlnlp 379 : | PSTPRTISRA SSFKRGFSS | | VSGHG-GSGK | | SMKSAINLGN | EKMS PPEZEN | KIPNNHIDAK | ISMOGSLNID |
| CaSlnlp 380 : | KYKKEKI | . NSVNSNSPTS | GSGSGSGSGS | GSGSGSRANS | | | | SSADQSLSLD |
| ScSlnlp 478: | LLGPHSLRHN DTDRSSNRSI | | | | | | | |
| CaSlnip 428; | TGKRNS INSSSFS | | KDELTELSEA | | | | IQAEAANEAK | |
| ScSlnip 578 : | LRTPLNGILG MIAISMEET | VNKIRNSLKL IFRSGELLLH | ILTELLTYSK | NVLQRTKLEK | RDPCITDVAL | QIKSIFGKVA | XDQRVRLSIS | LPPNLIRIMV |
| | LRTPLNGILG MISIAMERE | | ******* ** | * * * **** | | • ••• • • | **** * | •• • |
| | LMGDSNRIIQ IVMNLVSNAI | | | | | | | |
| | ******** | ******* * * ****** | * * * * ** | • • | | | | |
| Casinip 621 : | IYGDSNRIIQ IVMNLVSNSI | KPTPVDGSVS VSPKLLGEYD | HERSKKLDYK | RVCILNDSSS | STVAVPPPTP | PSDTKPNPKP | KSTPTPKPDP | TRSHLVDHAN |
| ScSlnlp 732 : | | | | | SNURKS | | v | DLESSATS-L |
| Caslnip 721 : | RSANTTSPLT PVRKPTNQTI | MKSITHNVTK QHHKIRKKK | TNUNLHOUNN | NNKNDNSDFL | MARKLEGSHK | FNNINDEELS | PIAIERNIDK | YLTSSADSDN |
| ScSlmlp 769: | GSNRDTSTIQ EEIT | ********* | KRNTVANE | -SIYKKVNDR | | DDVS- | s | IVSTTTS |
| CaSlnlp 821 : | ISVITLSTVQ YETTIFESQE | KSKPLPALPV DAKPOVSGKI | DENDVINDEDP | SGGSIKDODS | edtinekogi | SSSPSSSSSS | NEKÇENSPRS | NOSTIVIVIR |
| ScSlnlp 817 : | | APGSD DESGGN | | | | | | |
| CaSlnlp 921: | | KPEYDSNMSN NEIVKNORVY | | | | KVFEPFVQGD | | |
| ScSlnlp 901 : | ANNHHGINKL ESKVÇVÇSKI | | | | | svaks | iksrostssv | ATPATNRSSL |
| CaSlnlp 1021 : | ATMOHGTLTL KSTIGKGST | | EFCEDEFNPA | AKINRKVAFE | DGDIDTESQQ | OENPSSEEDT | QGDRNVQSST | SSSPPN-SSS |
| ScSlnlp 983 : | TNDVLPEVRSKGKHE | THUVGNPHING REEKNIDNOGL | EQLQEKNIK- | PSICLTGAEV | NEONSLSSK- | | | |
| CaSinip 1120 : | TDSALPASDS SOIGGINKS | * ** TTSHONNEDI NAKERIITNS | SASSTKOTKR | | | LTLTIDKPSL | ftrgstgtan | SCTTSSHSDK |
| ScSlnlp 1061 : | PPLOSTGTAT SSRNIPTVKI | DOKNETS-VK ILVVEINHVN | QEVINOUNL 1 | EGIENIELAC | | | | |
| CaSlnlp 1220 : | KILVETETT TITTTETIMI | | OSATZMATAĞ I | | ngakaidevk | | LIFIOVORDE | |
| ScSlnip 1160 : | | NIKECLESCH NGFLSKPIKR | | CAAYQGKIQW | - — — - к | - | | |
| CaSlnlp 1320 : | RKNLQYNKPI IALTAFADES | NVKECINSCH SGFITKPISK | INIKKVLVEP | LSNÉVVT | 5 | | | |
| - | | | | | - | | | |

Fig. 2. For legend see facing page.

there are some structural differences between the two proteins. As mentioned above, N. crassa Nik1p contains six repeats of 90 aa that always start with tryptophan, but the first repeat in CaNik1p begins with glutamic acid

and the fourth repeat observed in the N. crassa Nik1p is absent from CaNik1p (Fig. 2b). The regions from amino acid residues 490 to 641 and from 886 to 990 of CaNik1p share a high degree of sequence identity with the sensor

| Nemikia 1: Medgytaal aalumsiand pattotssia estimbilog tytesedies elsauvirie qletaalaas pumbetura ptolessot lessettoa canalaje 1: M-mpt | (b) | | | | | | | | | | | |
|--|-----------|--------|-------------|------------|-------------|---------------------|-------------|------------|------------|-------------|-------------|-------------|
| NCHIALD 101: RYPHIPRON PIDEALEGIR DATOROGAL DESCRIBATION NAQLICOTOR QUALATICO ENVATABREL ESTRONOMO QUALETORI VINAMARDIA CAMIRID 33: "LICENTE DESCRIBATION DESCRIBATION DESCRIBATION NAQLICOTORI ESTRONOMO QUALETORI VINAMARDIA CAMIRID 201: ROVERNESVEN DESCRITTORI INTROQUIO PRESENENZA ROVIEGILOS QAQUISOVICE MESINIMAN MAGALITORIS ELANTIMAN ACCLINACIDE CAMIRID 86: ROVERNITURI DESCRIVATI DITHOCOLOF PRESENENZA ROVIEGILOS QAQUISOVICE MESINIMAN MAGALITANIA ROLLINATION REMIRID 301: PARCELLOLO RITHOMOCOL RITASEVINA ARDAVITEGILO GAQUISOVICE MESINIMAN MAGALITANIA VARIBULATIVA VARIBULATI VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATI VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATIVA VARIBULATI VARIBULATI PARIBURATI PARIBURATI PARIBURATI PARIBURATI PARIBURA | NcNiklp | 1: | | AALVKSLAVD | PATTQTSGLR | | YTREKGDLER | | | | | |
| CANILLED 33 : "LIA E | CaNiklp | 1: | MNPT | | -KKPRLSPMQ | PS | | VFEI- | <u>LS</u> | DP | ELYSQHO | : HS |
| CAMINID 33 : ********************************* | NcNikip | 101 : | RYPAPLPRIC | | | | | | | | | |
| CANIKID 86 IXVELITIVEN DEPILIAVET INTRODUCT FARSYTNIAT EV-ANGELIG QUIDICSVGI MESIATONINI NAIALINIQUE ELADVERAVA REDLESERIND NERIKID 301: PANGELIGIQ QTINITWOOL RIVASEVIRVA ARDVOTEGIL GGGALVERVO GAMBELTINIV NANABALITIQ VERLIAVITA VANGLIGURV QASCRGEITE CANIKID 185: NAGCELIGIQ RITITMONDOL RIVASEVIRVA ARDVOTEGIL GGGALVERVO GAMBELTINIV NANABALITIQ VERLIAVITA VANGLISTON TACKGELID NENIKID 185: NAGCELIGIQ RITITMONDOL RIVASEVIRVA RADVOTEGIL GGGALVERVO GAMBELTINIV NANABALITIQ VERLIAVITA VANGGISTON TACKGELID NENIKID 285: LULTINGAND GLQGAREVI KLAREVATIG ILGOGARVOD VOGTREBLER MANGAORILI TAVARGALIKA KLOVENOGEI LULGUTUTUM LUCKTININA RIVASEVIRVA TAVARGALIKA KLOVENOGEI LULGUTUTUM LUCKTININA RIVASEVIRVA TAVARGALIKA KLOVENOGEI LULGUTUTUM LUCKTININA RIVASEVIRVA VARIANGARI KLOVENOGEI LULGUTUTUM LUCKTININA RIVASEVIRVA VARIANGARI KLOVENOGEI LULGUTUTUM RIVASEVIRVA GARRANGA SILVENARE SILVE | Canikip | 33 : | | | | | | | | | | |
| ANNIALD 46: UNIXULTIVEN DEPLIABELL INTRODUCT FREVITWAT EV-ANGELIC QUORDISCI MELATININI MALMINIQUE ELADUTRAVA RODISCRININ NICHIALD 30: PANGELICUQ CIDITANDOL RIPASEVITAV ARDVOTIGOLI GEGALVERUO GAGRETININ NICHIALDITO VERDINATTA VANGDISTAV QUARRETIE CAMINID 18: HAGGELICUQ STIRMMODE RIPASEVITAV ARDVOTIGLI GEGALVERUO GAGRETININ NICHIALDITO VERBINAVITA VANGDISTAV TADEKGEILD NICHIALDI 18: HAGGELICUQ STIRMMODE RIPASEVITA REDUCCIOLI GEGALVERUO GAGRETININ NICHIALDI VANGDISTAV TADEKGEILD NICHIALDI 18: LEXITININDO GLOGFARRAT KLARENGTEG RUCCANTAND VOCTORDILTE MARGAMARILI TAVRELARVI TAVARGDITA KLOVEVOGEI LOLAVITUMIN CARILLA 19: LEXITININDO RUCCATANO COLLOCARUM UNDERNOQUE MARGAMARILI TAVRELARVI TAVARGDITA KLOVEVOGEI LOLAVITUMIN CARILLA 19: VERLOTTARE VERVAREVET DELLOCARUM UNDERNOQUE MARGAMARILI TAVARGITAT TAVARGDITA KLOVEVOGEI LOLAVITUMIN CARILLA 19: VERLOTTARE VERVAREVET DELLOCARUM UNDERNOQUE MARGAMARI TERMININGO MELANGAMI GOLIDARICA SURVEYARAS ELILLASTIN NAMORILITA CARILLA 19: VERLOTTARE VERVAREVET DELLOCARUM UNDERNOCA DELLOCARUM CARILLA 19: VERLOTTARE VERVAREVET DELLOCARUM UNDERNOCA DELLOCARUM UNDERNOCA DELLOCARUM CARILLA 19: VERLOTTARE ALGUMINO CARILLA 19: MARGAMARIS ELILLASTIN NAMORILITA QUARRICANO DELLOCARUM DELLOCARUM DELLOCARUM UNDERNOCA DELLOCARUM UNDERNOC | NcNiklp | 201 : | | | | | | | | | | |
| CANIKID 185 : HAGGEILQLQ RTININWOOL RITAFEVSKV ARDUGVLGIL GOGALIENVE GIMERLTENN NAMALELTIQ VERILANVITA VANGDLSKKV TADCKGEILD NCHIKID 401 : LOXITISAND GLQGFAREVI KIARENGIEG RIGOGATHAD VQCTMERLIE MANSADARIT TOVRELAKVI TAVAKGLITK KIGVEVGGEI LOLANTININ CRHIKID 285 : LULTINGNUD RIGHFALAVI TILSERWRILG RIGOGATHAD VQCTMERLIE MANSADARIT TOVRELAKVI TAVAKGLIJK KIGVEVGGEI LOLANTININ NCHIKID 501 : VURLGITAFE VSKNAREVGI DGILGOGAQU DENEGREGIL TERMITHASEN LITSGVAGIST VIQALAKGEN SEKIEVERKE EILILEGTIN NAMGRISTIC CANIKID 385 : VUSLGUFASE VSKNARDVGI NGKGIGAQU NCHIKID 385 : VUSLGUFASE VSKNARDVGI NGKGIGAQU NCHIKID 401 : NEVGRVAKID GVGGIGGGA DANGLAGREK EITIDANING BELIZAQVANF GDITHAATDG DFTRIJVENA SCHOOLAKKI NQAVYALRO SIGRHTGARE CANIKID 401 : NEVGRVAKID GVGGIGGGA DANGLAGREK EITIDANING BELIZAQVANF GDITHAATDG DFTRIJVENA SCHOOLAKKI NQAVYALRO SIGRHTGARE NCHIKID 701 : AARLANGKIS BELANGSBEI RITANIGIGH TQLILDTELT GYGRADGATV NGLANSILITI IDDILDISKI RABBNITEEI PYTHRITVEN ALKTILANKET CANIKID 403 : AARLANGKAS BELANGSBEI RITANIGIGH TQLILDTELT GYGRADGATV NGLANSILITI IDDILDISKI RABBNITEED PYTHRITVEN ALKTILANKET CANIKID 503 : EKOLDITYGO DESPENDLIG DEFERGYUL NILAGAJIRFT REGKYS-VEV KESDRONIDS KLLLEKUVIS TGLGIRDIGL GLIFDTFQA DESTRUTGG NCHIKID 504 : TGLGLISISKI LINGHGGAN VKSENGKOSK FFFTUVALA NEDISLIANG LINFYSSKOL FIDRIGTIGN PELADOL.—BELGLUFIV VUSCENDALE CANIKID 507 : TGLGLISISKI LINGHGGAN VKSENGKOSK FFFTUVALA NEDISLIANG LINFYSSKOL FIDRIGTIGN PELADOL.—BELGLUFIV VUSCENDALE CANIKID 507 : RARAAGARFY DVIIVESIED ARRIESENSE FYFTUVISKA NEDISLIANG LINFYSSKOL FIDRIGTIGN TECQLIDIGN GIVERNATIL FERDENSE FERDENSE FIRMINGEN VKSENGKOSK FFTTUVISK NEDISLANG TRUTTERGE CLOUGHER TECQLIDIS ATTERLESSE ISQUEDESSA YKILLARDAL NCHIKID 506 : VKGRLAVKIL EKYDHVUVU GNEBEAVEAV KROGTOVILM DVGRFTROG PETARLIFEE ——SEAG SCRIPTIAL ARABODREK COAQUERY NCHIKID 508 : VKGRLAVKIL GUIDKGEN VENEZUSAN VKROGTOVILM DVGRFTROG PETARLIFEE ——SEAG SCRIPTIAL ARABODREK COAQUERY NCHIKID 509 : SCHLARDAL GUIDKGEN FILMINGEN REGEROVAN TORRUMAN ——STROGSDE GSVERHIGT PROGSVERGE SSSTUTATE DPLSDARAE. CANIKID 509 : SCHL | CaNiklp | 86 : | | | | | | | | | | |
| CANIKID 185 : HAGGEILOLO RITATHWOOL RITATEVSKY ARDVOYLGIL COOLIENVE GIGGELITAN NAMADALITO VERLANVITA VANGDLSKAY TALCKEELID NCHIKID 401 : LAGITISSMO QLOGFAREVI KLAREVOTEG RIGGOATVED VQCTARDLIE MANAGAMNIL TOVRELAKVI TAVAKGLIK KLOVEVQGEI LDLAVITHYM CONIKID 285 : LAGITINGMO RIGGEALANY TISSENGTILO ILGOGANQO VECANKQVIE NAMINATHI NOVESIATVI TAVAKGLISO KLUHAQGEI LQLAVITHYM NCHIKID 501 : VUBLOTFARE VSKVAREVOT DOTIGGOAQV DINEDDEGIL TERMINASIA LTSQVRGIST VIQAIANCHI SKILIVERAG EILILECTIN INMORLSITC CANIKID 385 : VUBLOFFARE VSKVAREVOT DOTIGGOAQV DINEDDEGIL TERMINASIA LTSQVRGIST VIQAIANCHI SKILIVERAG EILILECTIN INMORLSITC CANIKID 385 : VUBLOFFARE VSKVAREVOT DOTIGGOAQV DINEDDEGIL TERMINASIA DITUANTO DETRIVEVEA SCHOLEUGK INQAVANIAD SIQRINGARE CANIKID 401 : NEVGRVARDV GVUCINGGOA DINGLIGGAQV NCHIKID 401 : NEVGRVARDV GVUCINGGOA DINGLIGGAQV NCHIKID 402 : ABELANCIUS EFLANGSEI RITHMOILGA TQLILDIELI QYQREMISIV INLANSLIJI IDDILDIGKI EARRWITEEI PITHATIVEN ALKILIVEET CANIKID 403 : ABELANSANS EFLANDSSEI RITHMOILGA TQLILDIELI QYQREMISIV INLANSLIJI IDDILDIGKI EARRWITEEI PITHATIVEN ALKILIVEET CANIKID 503 : ENGLILTIVO DISSPENBLIG DEFREQUIL NIMORANGTE REGEVISITIQ KASSVOCSTE EVALEFOVED TIGGIEROKI GLIFUTYQA DOSTRAFGO NIKID 503 : ENGLILDIVO DISSPENBLIG DEFREQUIL NIMORANGTE REGEVISITIQ KASSVOCSTE EVALEFOVED TIGGIEROKI GIFTUTQA DOSTRAFGO NIKID 504 : TIGGISISKE LURIHERDUM VINSENGKESK PEFTUVUSES NIKUTROTEO LIPPISHOVE FULDENING PELARIM. — BULLUPTU VISENGALE NIKID 507 : RABANGQAPI DULINDISED ABELESVODE KILEVINE KIRTUTQUED LIPPISHVU F.—VSTENT GEELDVIROG TIEGLIPTI V.—RR—TE NIKIKID 507 : RABANGQAPI DULINDISED ABELSVODE KILEVINE KIRTUTQUED LIPPISHVU F.—VSTENT TROQUIDLAS ATTRILESS ISONOBENK YATILLAEDM NIKID 508 : VNQLAVKIL EXTREVITY ORGERAVEAN KRRUPVILIN DVARTOGF EXTENDEDE TROQUIDLA ATTRILESS ISONOBENK YATILLAEDM NIKID 509 : VNQLAVKIL EXTREVITY ORGERAVEAN KRRUPVILIN DVARTOGF EXTENDEDE KRRUPUGLI TATRILIA AADSLUSGIK SPINTARKE DPLSRARAS. NCHIKID 509 : SURLOGRIN OTILICATIG OQLERGRE ELIRADAVT GORDNOMS ASQAAQHAAL REPLATEGIC FROGSOGG TES—REV QREATEDSI NCHIKID 500 | Neniklp | 301 : | | | | | | | | | | |
| NCHIKIP 401: LOXIDISHND QLQQFARSYI KIARSUTEG RLCQATARD WQTARDLTE MONSONNLT TOWRSLAWY TAVARGDLTK KIGVEVOGEI LOLANTINTEN CRHIKIP 285: UKLTINGMO RLQBFALAVI TLSREWOTLG ILGQQAVQD VECHARQVIE MONSONNIT TOWRSLAWYI TAVARGDLTK KIGVEVOGEI LOLANTING NCHIKIP 501: VURLGTFAYE VSKVAREWOT DOTLGGQAQV DAWELDERGU TENWINGEN LISQVARGIST VIQALANDIM SKRIEVEAKG EILLIKSTIN NAVDRISTIC CANIKIP 385: VUSLQLFASE VSKVAQUODI NKHLGIQAQV NCHIKIP 601: NEVQRVAKOV GVOGINGQA DAWGLEGEME EITTOWNING BELIZAQVALV GDITHAATDG DFTKLVEVEA SGENGALTIK INDAVINGAS SIQRUYQARE CANIKIP 601: NEVQRVAKOV GVOGINGQA DAWGLEGEME EITTOWNING BELIZAQVALV GDITHAATDG DFTKLVEVEA SGENGALTIK INDAVINGAS SIQRUYQARE NCHIKIP 701: AABLANGISS BYLANGSEEI RITHAGIIGM TQLTLDTIELT QYGRABLAVI NILANSLUTI IDDILDISKI EDABNITEEI PYTLAGTVEN ALATLAVKET NCHIKIP 701: AABLANGISS EPLANGSEEI RITHAGIIGM TQLTLDTIELT QYGRABLIV NILANSLUTI IDDILDISKI EDABNITEEI PYTLAGTVEN ALATLAVKET NCHIKIP 801: ENFLDLITAV DISVPINVUG DEFRLAQIIL NILADBAIRFF BHGEVSLITQ KASSVQCSTE EVALEVVOS TGIGLFAUR DLIFUTYQA DESFRAVGA CANIKIP 593: ENGLDLUTYQC DESFRANIG DEFRLAQVIL NILAGDAIRFT REKKYS-VEV KREDNINIES KILLEVCVO TGIGLEDKI GLIFUTYQA DESTRATOG BCHIKIP 901: TGIGLISISER LVHLHKODOM VASSYOKISK FFFTXVALA NDDISLIJANG LAPPYNSKAVL FILANGING FELANDA. — HULLIVFIV VUSERIPALE CANIKIP 997: RARANGAPT DVILVESIED ARBRITSSEN KYTHAVUMI SIFQLANGVU IILGISSYNV TEOSITOLAS ALTRALESSI SIGNEDESVA YELLARDINI NCHIKIP 996: VNQRIAVKIL EKYBNOVIVV GREERAVENE KURNYDING FENENDERF ETERLIGDE KUSHFILMI ANDMIGREK SIGNEDESVA YELLARDINI NCHIKIP 996: VNQRIAVKIL EKYBNOVIVV GREERAVEN KROOPDVILM DVGRVOKOF ENTERIOGE KUSHFILMI AADSLVSILK SPELVARRE DPLSRAMAS. CANIKIP 981: SUULOGRILM QULBURGEN RUSHFANDAY GGRRUNGAF ENTERIOGE KUSHFILMI AADSLVSILK SPELVARRE DPLSRAMAS. CANIKIP 982: SUULOGRILM QULBURGEN RUSHFANDAY GGRRUNGAF ENTERIOGE GEVENNIGET FROGSVEGGG TSSRPV QRBANTEGSI NCHIKIP 983: SUULOGRILM QULBURGEN NGERFANDAY GRRUNGAR SEGENDAMI RPPLATEGIT PROGSVEGGG TSSRPV QRBANTEGSI | ~ | | | | | | | | | | | |
| CANIKID 285: KALTINGNUD REGNETALAYI TESREWITED ILGOGONAYOD VEGAMKQITE MANIMATHET MANISLATVI TAVANGOLSO KIDVANGGEI LQURATINON NEMIKID 501: VERLOTTARE VSKVAREWET DOTLOGONAYO DAWEDWOOD LEDWANDARE LESGANGUST VIQALINKEM SRETEVANG ELLILASTIN NAMDRESIEC CANIKID 385: VESLOLFASE VSKVAQUWGI MSKLGIQAQV NEMIKID 601: NEVQRVARDA GVOGDHOGON DAWEGAGEN ELITDAVIDA BELIZAQURAF COLINANTOG DETRUVEVEN SCHOOLIJOK IMMANIKO SIQRNIQARE CANIKID 415: | | | | | | | | | | | | |
| CANIKID 285 : UKITINGNUD RIGHFALAVT TISRENGTIG ILGOGANNOG VECANROVTE NARINGTHIT NOVESIATUT TAVANGDISQ KILVINGGEI LQUAVIDHOR NONIKID 501 : VERLOTFAFE VSKVARENGT DGTLGGGAQV DAVEGREGED. TENNYHMASH LTSQURGIST VTQAIANGIM SRKIEVEARG EILLIKETIN NAVDRISTIC CANIKID 385 : VESLOLPASE VSKVAQUNGI NCKLGIQAQV NONIKID 601 : NEVGRVARIU GVUGINEGGA DVASILGEREK EITTOWNINA BELTAQVIRAF GDITHAATDG DFTRIJVERA SGENDELKOK INDAVMERAD SIGRMYQARE CANIKID 601 : NEVGRVARIU GVUGINEGGA DVASILGEREK EITTOWNINA BELTAQVIRAF GDITHAATDG DFTRIJVERA SGENDELKOK INDAVMERAD SIGRMYQARE CANIKID 601 : AMELANKIKS EFLANDSBEI RTHRAGIIGM TQLILDITELT GYGRENINIV NSLANSLUTI IDDILDISKI EMBRUVLGGI EFTILAGUVER ALKTLAVKET CANIKID 601 : AMELANSAKS EFLANDSBEI RTHRAGIIGM TQLILDITELT GYGRENISIV NGLANSLUTI IDDILDISKI EMBRUVLGGI DFSLROVVE ALKTLAVKAT CANIKID 601 : EKFLOLITYRV DHSVPIHVVO DSFRIRQIIL HUXDALITET GYGRENISIV NGLANSLUTI IDDILDISKI EMBRUVLGGI DFSLROVVE ALKTLAVKAT CANIKID 601 : EKFLOLITYRV DHSVPIHVVO DSFRIRQIIL HUXDALITET GYGRENISIV NGLANSLUTI IDDILDISKI EMBRUVLGGI DESTROVVE ALKTLAVKAT CANIKID 601 : TGLGISISKE LINUMERGOW VKSEYGKGSK FFTEVVELA NEDISLITAG LAIPVVSKYLD TGLGIERUKL GLIFDTFCQA DGSTTRIPOG CANIKID 602 : TGLGISISKE LINUMERGOW VKSEYGKGSK FFTEVVELA NEDISLITAG LAIPVVSKYLD FLOKGRUNG PELANG. ——HULLIVPIV VUSERRPALE NENIKID 997 : KARAAGQAPY DVILVUSIED ARBIRSVEDP KYLDYUVLA NEDISLITAG LINUTSSHVIL FVSTERT GEELDVARD TIEGGIPTI VRHIE NENIKID 996 : VARLAVKIL EKYRHVYVV GASEAVEAV KROUPVVIM DVORPHOGF ENTAKUREY | NcNik1p | 401 : | LEKTINSMVD | QLQQFAREVT | KIAREVGTEG | RLCCOATVHD | VOCTWRDLTE | NVNCMAMNLT | TOVRELAKVI | TAVARGDLTK | | |
| CANIKID 385 : VUSIQUEASE VSKVAQUNGI NCKLGIQAQV NCNIKID 601 : NEVQRVARIUV GVDGIMSGGA DVAGLKGRAK EITTUVNINA MELIZAQVRAF GDITNAATDG DFIRLIVEVEA SGEMDELKKK INQAVVALED SIQRMQARE CANIKID 415 : | CaNiklp | 285 : | LIKLTINGHVD | RLONFALAVT | | | | | | | | |
| CANIKID 385 : VOSIGLEASE VEKVAQUMGI NEKIGIQAQV NEMIKID 601 : NEWQRVARID GVDGIMSGOA DWAGLKERNE EITTDWININA BRILIZAGVRAF GDITNAATIG DFTRITVERA SCHEDLIGKE INQUVANLED SIQRNIQARE CANIKID 415 : | NcNik1p | 501 : | | | | DWYEGK <u>WK</u> DL | TENVNIMASN | LTSQVRGIST | VTCAIANCEM | SRICLEVEAKG | EILILKETIN | NHVDRLSTFC |
| CANIKID 415: | Canikip | 385 : | | | | | | | | | | |
| CANIKID 415: | NcNiklp | 601 : | NEVQRVARDV | | | | | | | | | |
| Nenikip 701: AARLANKIKS EPLANGSEL RIPHNGLIGM TOLILDIDLI GYGREMLNIV NSLANSLLTI IDDILDISKI RARRAVIREI PYTRGTVFN ALKILAVKAI CANIKIP 493: AARLANSAKS EPLANGSEL RIPHNGLIGM TOLILDIDLI GYGREMLSIV KNLANSLLTI IDDILDISKI RARRAVIREI PYTRGTVFN ALKILAVKAI Nenikip 801: EKFLOLTYRV DESVPIHVUG DEFRLEGILL NLAGNALKET REGEVSLATIQ KASSVQCSTE EVALEFVVSD TGIGIPADEL DLIFDIFOGA DGSHTREFGG CANIKIP 593: EKOLDLTYGC DESFPENLIG DEFRLEGILL NLAGNALKET REGEVSLATIQ KASSVQCSTE EVALEFVVSD TGIGIPADEL GLIFDIFOGA DGSHTREFGG NENIKIP 901: TGIGLSISKE LUNLMSGUNW VKSEYGRGSK FFFTXVVRLA NDDISLIARG LEFVENSVL FIDKORKOKS PELAKEL.——HGLGLVPIV VDSERNPALE CANIKIP 692: TGIGLSISKE LUNLMSGUNW VKSEYGRGSK FFFTXVVRLA NDDISLIARG LEFVENSVLV FIDKORKOKS PELAKEL.——HGLGLVPIV VDSERNPALE NENIKIP 997: KARAAGQAPY DVIIVDSIED ARREKSVEDF KYLPIVLISK SIRVITROTED LLPFSSHYVL FVSTEMT GEELDVIROG TIELGLIPTI VRMTE CANIKID 784: DATLTEFVKY DIDMIDSIEI AKKIRLISEV KYIPLVLVIM SIPQLWERVC IDLGISSTAN TECSITOLAS ALIPALESES ISONSDESVA YKILLARDAL NENIKIP 1096: VNORLAVKIL EKYHNVTVV GROERAVEAV KRIKDPUVIM DVORPINGGF ENTAKTREVERSIG SQRTFITALT ARAMGDREK CIQAGNDEVL CANIKID 884: VNOKLAVKIL EKYHNVTVV GROERAVEAV KRIKDPUVIM DVORPINGGF ENTAKTREVE | Calli bia | 415 . | | | | | | | | | | |
| Canikid 493: AARLANSAKS EFLANGSHEI RTFLNGIIGN TQLSLDTELT GYGREMLSIV KNLANSILITI IDDILDISKI BARRMIVEQI DESLRGTVEG ALKTLAVKAI NCNIKID 801: EKPLDLIYAV DHSVPIHVUG DESPREQIIL NLAGNAIKFT EHGEVSLATIQ KASSVQCSTE EYALEFVVSD TGIGIPADKL DLIFDITQGA DGSMTRUPGG CANIKID 593: EKOLDLIYQC DSSPPINGLIG DESPREQVIL NLAGNAIKFT KEGKVS-VSV KKSDKWVLDS KLILEVCVSD TGIGIPADKL GLIFDITQGA DGSMTRUPGG NCNIKID 901: TGLGLSISKE LVNIHAGDUM VKSEYGKGSK FFFTCVVRLA NDDISLIAKG LAPPKSHQVL FIDKGRICHG PELANGL | Canakap | 475 : | | | DVDGLWK | RITSMANIMA | SNUTSQVRAF | AQITAAATDG | DFTRFTTVEA | SGEMDALKTK | INOMVENURE | SLORNTAARE |
| Canikid 493 : AAELANSAKS EPLANMSHEI RTPLNGIIGN TQLSLDTELT QYQREMLSIV KNLANSLLTI IDDILDISKI ENRMYVEQI DFSLRGTVFG ALKITAVKAI Nenikid 801 : EKPLOLTYKV DHSVPINVVG DSFRLRQIIL NLAGNAIKPT EHGEVSLATIQ KASSVQCSTE EYALEFVVSD TGIGIPADKL DLIFDITQQA DGSMIRKFGG CANIKID 593 : EKOLDLTYQC DSSFPUNLIG DSFRLRQVIL NLAGNAIKPT KEGKVS-VEV KKSDKNVLDS KLLLEVCVSD TGIGIPADKL GLIFDITQQA DGSMIRKFGG Nenikid 901 : TGLGLSISKR LVRLMOGDVM VKSSYGKGSK FFFTCVVRLA NDDISLIAKQ LNPYKSHQVL FIDKGRIGMG PELAKNLHGLGLVPIV VDSERNPALE CANIKID 692 : TGLGLSISKQ LIHLMOGEIM VISSYGGSSN FYPTVCVSPS NIRYTRQTEQ LLPFSSHYVL FVSTEMT GEELDVLRDG IIELGLIPII VRNIE NENIKID 997 : KARAAGQAPY DVIIVOSIED ARRLRSVODP KYLPIVL-LA FVVHVSLKSC LDLGITSYMT TPQQLIDLGN GMVPALENRA TPSLADNIKS FEILLAEDNI CANIKID 784 : DATLTEPUKY DIDMIDSIEI AKKURLISEV KYIPLVLNIH SIPQLMENCU IILGISSYAN TFCSITDLAS AIIPALESRS ISONSDESVR YKILLAEDNI NENIKID 1096 : VNGRLAVKIL EKYHNVVTVV GNGERAVEAV KRRKPDVILM DVQMPINOGF ENTAKIREYE | NcNikip | 701 : | AAELANKIKS | EPLANNSHEI | RTPMNGIIGM | TOLTLOTOLT | OYOREMINIV | NSLANSLLTI | | | | |
| Canikip 593 : Ekoldityoc desprinlig defrirqvil nimenaikft keckve-vev kkedknylde kullevoved teigiekoki glirdiroga desttrirog Nenikip 901 : teigleiskr lvalmagdum vaseygrgek ffficvarla nddieliarg lapyrekovil fidkortoke pelakalheiglypiv viserapale Canikip 692 : teigleisko lihimageim viseygreen fyffuover nikytroted lidpeshyvl fvetent geeldvirdd iielglipti vknie Nenikip 997 : karaaggapy dviivdeied arbirsvodf kylpivl-la fyvhvelkec lolgitsynt trodidien gwyralenka tesiadnyke fellaedny Canikip 784 : datliepvky diibideiel arbirsvedf kylpivl-la fyvhvelkec lolgitsynt trodidien gwyralenka tesiadnyke fellaedni Nenikip 1096 : vackavkil ekyhhvviv greeaveav krhudvim dvorpinggf eatakireye | Caniklp | 493 : | | | | | | | | | | |
| Canikip 593 : EKOLDITYOC DESPRENCIG DEFRICOUIL NLAGNAINFT NECKVE-VEV KNEDKNOLDS NLLLEWCVED TGIGLERONL GLIFDTFCQA DGSTTRUFGG NCNIKIP 901 : TGIGLSISKE LVNLHGGOWN VNSEYGRGSK FFFTCVVRLA NDDISLIARQ LNPYKSHOVL FIDKGRYCHG PRIARMHGIGLVPIV VDSERNPALE CANIKIP 692 : TGIGLSISKO LIHLHGGEIN VTSEYGSGSN FYPTVCVSPS NIRYTROTED LLPFSSHYVL FVSTEHT GEFLDVLRDG HELGLIPHI VRNIE NCNIKIP 997 : KARAAGQAPY DVIIVDSIED ARRIESVEDF KYLPTVL-LA FVVHVSLKSC LDLGITSYNT TPCQLIDLEN GNVPALERRA TPSLADNIKS PEILLAEDNT CANIKIP 784 : DATLTEPVKY DIDNIGSIEI ANGURLISEV KYIPLVLVHH SIPQLNERVC HILGISSYAN TFCSITDLAS AHPALESRS ISONSDESVA YKHLAEDNI, NCNIKIP 1096 : VNORLAVKIL EKYMHVVTVV GNGEBAVEAV KRUGPDVILM DVQMPINGGF ENTEKLROME KKSNPIDSL- TFRIPILALT ANAMMIDREK CIQAQMOEYL CANIKIP 1190 : SKPLQONHLI OTILKCATLG GQLLEKNERE ELTRAADAVT OGRRUNNYS ASQAAQHAAL RPFLATRGIT AADSLVSGLE SPSIVTADKE DPLSRARASL CANIKIP 983 : SKPLQONHLI OTILKCATLG GQLLEKNERE ELTRAADAVT GGRRUNNYS ASQAAQHAAL RPFLATRGIT AADSLVSGLE SPSIVTADKE DPLSRARASL CANIKIP 1290 : SEPNIBKAS | NeNiklp | 801 : | | | | | | | | | | |
| NCNIKIP 901: TGLGLSISKE LVNIMGGOW VKSEYGKGSK FFFTCVVRLA NDDISLIAKO LNPYKSHOVL FIDKORIGHG PELARALHGLGLVPIV VDSERNPALE CANIKIP 692: TGLGLSISKO LIHLMGGEIW VISEYGSGSN FYPTVCVSDS NIKYTROTED LLPFSSHYVL FVSTEHT GEELDVLRDG ITELGLIPTI VRNIE NCNIKIP 997: KARAAGQAPY DVIIVDSIED ARRLRSVDDF KYLPTVL-IA PVVHVSLKSC LDLGITSYMT TROQLIDLGN GMVFALERRA TPSLADMYKS PELLIAEDMT CANIKIP 784: DATLTEPVKY DIDMIDSIEI ARKLRILSEV KYIPLVLVHH SIPQLMGRVC IDLGISSYAN TROSITOLAS AIIPALESRS ISONSDESVR YKILLAEDML NCNIKIP 1096: VNORLAVKIL EKYGHVVTVV GNGEBAVEAV KRKRPDVILM DVQMPIMOGF EATAKIREYERSLG SQRTPIIALT AHAMMODREK CIQAQMDEYL CANIKID 884: VNORLAVKIL EKYGHSVEVV ENGLEAVEAI KRNKYDVVLM DVQMPIMOGF EATAKIREYERSLG SQRTPIIALT AHAMMODREK SLAKGMODYV NCNIKID 1190: SKPLOGNHLI OTILKCATIG GQLLEKNRER ELITRAADAVT OGRRDNOMYS ASQAAQHAAL REPLATRCIT AADSLVSGLE SPSIVTADKE DPLSRARASI CANIKID 983: SKPLOGNHLI OTILKCATIG GQLLEKNRER ELITRAADAVT OGRRDNOMYS ASQAAQHAAL REPLATRCIT AADSLVSGLE SPSIVTADKE DPLSRARASI NCNIKID 983: SKPLOGNHLI OXINKCIHNI NQLKELSHNS RGSDFAKKMTRNTFOSTTRQGSDE GSVEIMIGDT PROGSVEGGG TSSRPV QRRSATEGSI NCNIKID 1290: SEPNIDKAS | CaNiklp | 593 : | | | | | | - | | | | |
| Canikip 692 : Telgisisko liminggein visengson fyptvovode nikytroteo ilippishyvl fvstem geeldvirog itelgilpii vrnie Nonikip 997 : Karaagoafy dviivosied arrirsvoof kylpivi-la pvvhvsikso idigitsymt teodidion gavpalerra tesiadayks peiliaedat Canikip 784 : Datltepvky didmidsiei akkirilsev kylpivivim sipoingroc idigissyan teositolas alipalesre isonsdesva ykiliaedal Nonikip 1096 : Vnorlavkil ekymmytvv gageeaveav kregedviim dvompingge eatakireye | | | | | | | | | | | | |
| Nenikip 1997 : Karaagaay dviivdsied arriesvoof kylpivl-la pvvhvslksc lolgitsymt troolidlen gavpaleara tesladaiks feillaeimt Canikip 784 : Datltepvky didmidsiei akkirilsev kyipivlyhm sipolmorvo idlgissyan tecsitolas alipalesrs isonsdesva ykillaedal Nenikip 1096 : Vnorlavkil ekyhmvytvv gagepaveav krokpovilm dvompingge eatakireve | NCN1K1p | 901 : | TGLGLSISKR | EVNUMGGOVW | VKSEYGKGSK | FFFTCVVRLA | NDDISLIARQ | | | | | |
| CANIKID 784 : DATLTEPVKY DIDMIDSIEI AKKURILSEV KYIPLVLVHM SIPQLMERVC IULGISSYAN TECSITOLAS AIIPALESES ISONSDESVA YKILLAEDAL NCNIKID 1096 : VNQKLAVKIL EKYMMVVTVV GNGEBAVBAV KRKKPDVILM DVQMPIMOGF BATAKIREYERSLG SQRTPIIALT AHAMMGDREK CIQAQMDEYL CANIKID 884 : VNQKLAVKIL EKYMMVVTVV GNGEBAVBAV KRKKPDVILM DVQMPVMOGF BATAKIREYERSLG SQRTPIIALT AHAMMGDREK CIQAQMDEYL CANIKID 1190 : SKPLQQNHLI GYILKCATLG GQLLEKORER BLITAADAVT GGRRINGMYS ASQAAQHAAL RPPLATRGLT AADSLVSGLE SPSIVTADKE DPLSRARASL CANIKID 983 : SKPLQQNHLI GYILKCIHNI NQLKELSHNS RESDFAKONTRNTFQSTIRQGSDE GSVEIMIGDT PRQGSVEGGG TSSRPV QRRSATEGSI NCNIKID 1290 : SEPNDRAS | Caniklp | 692 : | TCLCLSISKQ | LIHIMGGEIW | VTSEYGSGSN | PYPTVCVSPS | nirytroteo | LLPPSSHYVL | FVSTEHT | QEELDVLRDG | TTELGLIPTI | AMA1E |
| CANIKID 1996: UNDRIAVRIL EKYMHVVTW GNGERAVEAV KRIKPDVILM DVOMPIMOGF EATAKIREYERSLG SQRTPIIALT AHAMMIDREK CIQAQMDEYL CANIKID 884: VNORLAVRIL EKYMHVVTW GNGERAVEAV KRIKPDVILM DVOMPIMOGF EATAKIREYERSLG SQRTPIIALT AHAMMIDREK CIQAQMDEYL CANIKID 884: VNORLAVRIL EKOGHSVEVV ENGLEAYEAI KRIKYDVVLM DVOMPIMOGF EATEKIROME KRSNPIDSL- TFRTPIIALT AHAMMIDREK SLAKGMODYV NCNIKID 1190: SKPLOONHLI OTILKCATLG GOLLEKNRER ELITRAADAVT OGRRDNOMYS ASQAAQHAAL RPPLATRCLT AADSLVSGLE SPSIVTADKE DPLSRARASI CANIKID 983: SKPLOONHLI OXINKCIMNI NQLKELSHNS RGSDFAKONTRNTPOSTTROGSDE GSVEIMIGDT PROGSVEGGG TSSRPV QRRSATEGSI NCNIKID 1290: SEPNIDKAS | NcNiklp | 997 ; | KARAAGQAPY | DVLIVDSIED | ARRLRSVDDF | KYLPIVL-LA | | | | | | PELLAFINT |
| Canikid 884 : VNOKLAVRIL EKOGHSVEVV ENILEAYEAI KRRKYDVVIM DVOMPVMGGF EATEKIROME KKSNPIDSL- TFRIPIIALT AHAMIGDREK SLAKGMDDVV NCNIKID 1190 : SKPLOGNHLI OTILKCATLG GOLLEKNRER ELITRAADAVT OGRRDNOMYS ASQAAQHAAL RPFLATRGIT AADSLVSGLE SPSIVTADKE DPLSRARASL CANIKID 983 : SKPLOGNLIM OXINKCIHNI NOLKELSKNS RGSDFAKKNTRNTFOSTIROGSDE GSVEINIGDT PROGSVEGGG TSSRPV QRRSATEGSI NCNIKID 1290 : SEPNIDICAS | CaNik1p | 784 : | | | | | | | | | - | YKILLAEDNI. |
| CANIKID 884 : VNOKLAVRIL EXOCHSVEVV ENGLEAYEAI KROKYDAVIM DVOMPVMOGF ENTEKIROME KKSNFIDSL- TFRTPIIALT AHMIGDREK SLAKGHODYV NCNIKID 1190 : SKPLOONHLI OTILKCATIG GOLLEKNRER ELTRAADAVT OGRRINGNYS ASQAAQHAAL RPFLATROLT AADSLVSGLE SPSIVTADRE DPLSRARASI. CANIKID 983 : SKPLOONHLI OXINKCIHNI NOLKELSENS RESDFAKONTRVTPOSTIROGSDE GSVEIDHIGDT PROGSVEGGG TSSRPV QRESATEGSI NCNIKID 1290 : SEPNDERAS | NcNiklp | 1096 : | VNQRLAVKIL | EKYHHVVTVV | GNGEEAVEAV | KRKKFDVILM | DVQHQPINGGF | EATAKIREYE | RSLG | | | |
| Canikip 983 : SKPLKPKLIM OKINKCIHNI NOLKELSHNS RGSDFAKKNTRNTFOSTTROGSDE GSVEIMIGDT PROGSVEGGG TSSRPV QRRSATEGSI Nenikip 1290 : SEPHIDRAS | Caniklp | 884 : | | | | KRNKYDVVLM | DVQHPVMQGF | EATEKIROWE | KKSNFIDSL- | TERTPITALT | AHAMIGTOREX | |
| Canikip 983 : SKPLNDRILM OXINKCIHNI NOLKELSRNS RGSDFAKKNITRNTPOSTTROGSDE GSVEDNIGDT PROGSVEGOG TSSRPV QRRSATEGSI Nenikip 1290 : SEPNIDIGAS | NcNikip | 1190 : | SKPLQQNHLI | OTILKCATIG | | | | | | | | DPLSRARAST. |
| | Canik1p | 983 : | SKPLICPKLLM | OXINKCIHNI | NOLKELSKINS | | | | | | | QRRSATEGSI |
| Caniklp 1073 : TTISEOIDR | NcNiklp : | 1290 : | SEPNIDUCAS | | | | | | | | | |
| | Caniklp 1 | L073 : | TTISEQIDR | | | | | | | | | |

Fig. 2. (a) The amino acid sequence of 5. cerevisiae SIn1p (ScSIn1p) is compared with that of C. albicans SIn1p (CaSIn1p). Identical amino acids between ScSIn1p and CaSIn1p are marked by asterisks. Probable transmembrane regions of ScSIn1p and CaSIn1p are double-underlined. (b) The amino acid sequence of N. crassa Nik1p (NcNik1p) is compared with that of C. albicans Nik1p (CaNik1p). Identical amino acids between CaNik1p and NcNik1p are marked by asterisks. The first two amino acids of each repeat of about 90 aa in NcNik1p and CaNik1p are double-underlined. Amino acid sequences were aligned using the BLAST and FASTA programs. Predicted histidine kinase and receiver domains are indicated by bold underlining and dashed bold underlining, respectively. '+' represents the positions of the histidine and aspartic acid residues that correspond to the autophosphorylated histidine and phosphorylated aspartic acid residues of ScSIn1p. According to the report of Santos & Tuite (1995), the CTG codon is decoded as serine instead of leucine.

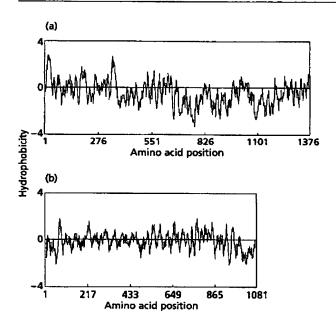


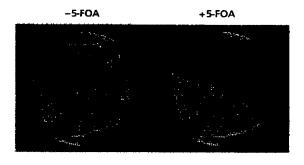
Fig. 3. Hydropathy plots of C. albicans SIn1p (a) and Nik1p (b) were calculated as described by Kyte & Doolittle (1982) using a window of 12 aa. It should be noted that two short domains with hydrophobicity indices of above 3 in CaSIn1p (a) are considered as transmembrane helices.

kinase and response regulator domains of twocomponent systems, respectively. Sequence comparisons with S. cerevisiae Sln1p and the BarA protein of Escherichia coli implicate the histidine at position 510 and the aspartic acid at position 924 as sites of phosphorylation (Fig. 2b).

DISCUSSION

We have isolated and sequenced a C. albicans homologue of ScSLN1. Like ScSln1p, CaSln1p possesses extracellular sensor, histidine kinase and receiver domains. When expressed from a multicopy plasmid, CaSLN1 overcame the growth defect of S. cerevisiae cells caused by the repression of ScSLN1. Since CaSln1p also shares significant sequence identity with the probable ATP-binding site within the kinase domain of ScSln1p, it should be able to autophosphorylate a histidine residue of the protein.

The receiver domain of CaSln1p was not necessary to rescue $sln1\Delta S$. cerevisiae cells. However, the mechanism of the complementation of ScSln1p by C-terminally truncated CaSln1p is not clear. In S. cerevisiae, the phosphate moiety at the autophosphorylated histidine residue in the kinase domain of Sln1p is transferred to an acceptor aspartic acid residue in the receiver domain of the same protein, then to a histidine residue in the downstream Ypd1p and finally to an aspartic acid residue of the further downstream Ssk1p, with both the



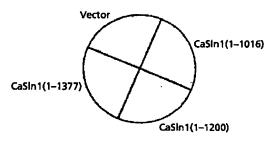


Fig. 4. Functional complementation of ScSLN1 by CaSLN1. S. cerevisiae pYES-SLN1 cells were transfected with YEp24T (vector) or YEp24T harbouring CaSLN1, which can encode full length CaSln1p [CaSln1(1-1377], or C-terminally truncated CaSln1p [CaSln1(1-1200) and CaSln1(1-1016)]. The transfectants were spread on plates with (+) or without (-) 5-FOA and were incubated for 3 d. The receiver domain is deleted in CaSln1(1-1200) and the receiver domain and half of ATP-binding site are destroyed in CaSln1(1-1016).

autophosphorylated histidine and receiver aspartic acid residues being essential for viability under low osmotic conditions (Posas et al., 1996). One possibility is that CaSln1p can bypass the phospho-relay to the receiver domain and to Ypd1p, and can function by directly phosphorylating Ssk1p.

In addition to CaSLN1, another gene, CaNIK1, was isolated and sequenced. The product of CaNIK1 is highly homologous to Nik1p of N. crassa, with regions that are highly related to the sensor kinase and response regulator domains of two-component systems. Although there is no apparent transmembrane helix in CaNik1p, we asked if CaNik1p has a similar function to ScSln1p. However, preliminary experiments did not support this hypothesis, because pYES-SLN1 cells harbouring CaNIK1 in a multicopy plasmid were unable to grow in the presence of 5-FOA or glucose. This result implies that CaNIK1 is functionally distinct from S. cerevisiae SLN1 and that CaSLN1 and CaNIK1 may not act in the same pathway. In fact, sequence homology between CaSln1p and CaNik1p is restricted to short regions encompassing the phosphorylated histidine and receiver aspartic acid residues. However, we cannot yet rule out the trivial possibility that the CaNIK1 gene failed to function in S. cerevisiae.

Unexpectedly, the homozygous casln1\Delta null mutation

32 48

sin1/sin1

80

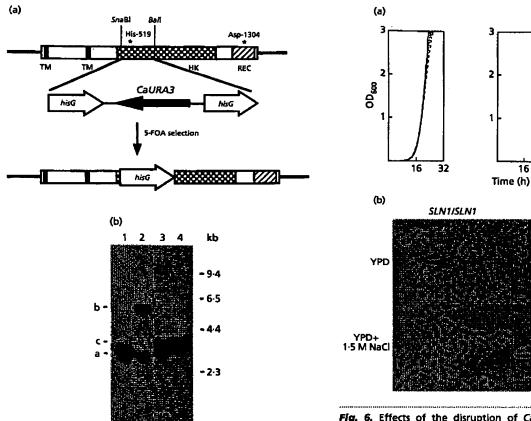


Fig. 5. Generation of the homozygous casIn1Δ null mutant strain. (a) The strategy for disrupting CaSLN1 is illustrated, with the SnaVBall region of CaSLN1 replaced by the hisG sequences. (b) Southern blotting of CaSLN1. Twenty-five micrograms of genomic DNA from wild-type CAI4 (lane 1), the hemizygous casIn1Δ mutant with URA3 (lane 2), the hemizygous casIn1Δ mutant without URA3 (lane 3) and the homozygous casIn1Δ null mutant (lane 4) was digested with Bg/II and Sall, fractionated on an agarose gel and hybridized with the 0-9 kb Rall-Sall fragment of CaSLN1. Bands derived from the CaSLN1 allele, the casIn1Δ::hisG-URA3-hisG allele and the casIn1Δ::hisG allele are indicated by a, b and c, respectively.

was not lethal in C. albicans. The homozygous casln1 Δ null mutant cells grew even under high osmotic conditions, but growth in the presence of 1-5 M NaCl was somewhat impaired by disruption of CaSLN1. This phenotype resembles that of the nik1 Δ null mutant of N. crassa (Alex et al., 1996) and also that of the hog1 Δ null mutant of C. albicans (Jose et al., 1996) and is suggestive of a CaSln1p function under high osmotic conditions. In contrast, the histidine kinase activity of ScSln1p is necessary under low osmotic conditions and is repressed under high osmotic conditions, leading to activation of the HOG1 MAP kinase in S. cerevisiae. Thus, it seems to be puzzling why a disruption of casln1 Δ caused a growth defect at high osmolarity. Although further experiments,

including the disruption of CaNIK1, are under way to

Fig. 6. Effects of the disruption of CaSLN1 on growth and morphology. (a) Effects of high osmolarity on the growth of wild-type CAI4 (SLN1/SLN1) (——), the hemizygous casIn1 Δ mutant (SLN1/sIn1 Δ) (....) and the homozygous casIn1 Δ null mutant (sIn1 Δ IsIn1 Δ) (-——). Cells of the indicated strains were cultured in YPD medium in the absence (left) or presence (right) of 1.5 M NaCl and the growth of the cells was monitored using a Biophotorecorder (Advantec). (b) Morphological change caused by the disruption of CaSLN1. Cells of wild-type CAI4 (SLN1/SLN1) and of the homozygous sIn1 Δ null mutant (sIn1 Δ IsIn1 Δ) were cultured in YPD medium in the absence (upper panels) or presence (lower panels) of 1.5 M NaCl. Photographs of cells from overnight cultures are shown. Bar, 10 μ m.

address this question, the absence of a NIK1 homologue in the S. cerevisiae genome suggests a divergence of osmosensing signal transduction mechanisms in fungi.

ACKNOWLEDGEMENTS

We thank B. L. Schneider for pMPY-ZAP, and S. Veronneau and A.-M. Sdicu for DNA sequencing. This work is supported in part by grant from the Ministry of Education and Science, Japan to T.O. Shigehisa Nagahashi and Toshiyuki Mio contributed equally to this work.

REFERENCES

Alex, L. A., Borkovich, K. A. & Simon, M. I. (1996). Hyphal development in Neurospora crassa: Involvement of a two-

component histidine kinase. Proc Natl Acad Sci USA 93, 3416-3421.

Baudin, A., Ozler-Kalageropoulos, O., Denouel, A., Lacroute, F. & Culin, C. (1993). A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic Acids Res 21, 3329-3330.

Bourret, R. S., Borkovich, K. A. & Simon, M. I. (1991). Signal transduction pathways involving protein phosphorylation in prokaryotes. *Annu Rev Biochem* 60, 401-441.

Brewster, J. L., de Valoir, T., Dwyer, D., Winter, E. & Gustin, M. C. (1993). An osmosensing signal transduction pathway in yeast. Science 259, 1760-1763.

Brown, J. L., North, S. & Bussey, H. (1993). SKN7, a yeast multicopy suppressor of a mutation affecting cell wall β-glucan assembly, encodes a product with domains homologous to prokaryotic two-component regulators and to heat shock transcription factors. J Bacteriol 175, 6908–6915.

Chang, C., Kwok, S. F., Bleecker, A. B. & Meyerowitz, E. M. (1993). *Arabidopsis* ethylene-response gene *ETR1*: similarity of product to two component regulators. *Science* 262, 539–544.

Fonzi, W. A. & Irwin, M. Y. (1993). Isogenic strain construction and gene mapping in Candida albicans. Genetics 134, 717-728.

Hua, J., Chang, C., Sun, Q. & Meyerowitz, E. M. (1995). Ethylene insensitivity conferred by *Arabidopsis ERS* gene. *Science* 269, 1712.

Jose, C. S., Monge, R. A., Perez-Diaz, R., Pla, J. & Nombela, C. (1996). The mitogen-activated protein kinase homolog *HOG1* gene controls glycerol accumulation in the pathogenic fungus *Candida albicans. J Bacteriol* 178, 5850-5852.

Kyte, J. & Doolittle, R. F. (1982). A simple method for displaying the hydrophobic character of a protein. *J Mol Biol* 157, 105–132.

Maeda, T., Wurgler-Murphy, S. M. & Saito, H. (1994). A two component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* 369, 242-245.

Mio, T., Yabe, T., Sudoh, M., Satoh, Y., Nakajima, T., Arisawa, M. & Yamada-Okabe, H. (1996). Role of three chitin synthase genes in the growth of Candida albicans. J Bacteriol 178, 2416-2419.

Nagahashi, S., Nakayama, H., Hamada, K., Yang, H., Arisawa, M. & Kitada, K. (1997). Regulation by tetracycline of gene expression in Saccharomyces cerevisiae. Mol Gen Genet 255, 372–375.

Ota, I. M. & Varshavsky, A. (1993). A yeast protein similar to bacterial two component regulators. Science 262, 566-569.

Parkinson, J. S. & Kofold, E. C. (1992). Communication modules in bacterial signaling proteins. Annu Rev Genet 26, 71-112.

Posas, F., Wurgler-Murphy, S. M., Maeda, T., Witten, E., Thai, T. C. & Salto, H. (1996). Yeast HOG1 MAP kinase cascade is regulated by a multiple phosphorylating mechanism in the SLN1-YPD1-SSK1 'two-component' osmosensor. Cell 86, 865-875.

Sambrook, J., Fritsch, E. F. & Manlatis, T. (1989). Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sanglard, M., Ischer, F., Monod, M. & Bille, J. (1997). Cloning of Candida albicans genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. Microbiology 143, 405–416.

Santos, M. A. S. & Tulte, M. F. (1995). The CUG codon is decoded in vivo as serine and not leucine in Candida albicans. Nucleic Acids Res 23, 1481–1486.

Schneider, B. L., Steiner, B. T., Seufert, W. & Futcher, A. B. (1996). pMPY-ZAP: a reusable polymerase chain reaction-directed gene disruption cassette for *Saccharomyces cerevisiae*. Yeast 12, 129–134.

Stock, J. B., Lukat, G. S. & Stoch, A. M. (1991). Bacterial chemotaxis and the molecular logic of intracellular signal transduction networks. *Annu Rev Biophys Chem* 20, 109–136.

Wilkinson, J. Q., Lanahan, M. B., Yen, H.-C., Glovannoni, J. J. & Klee, H. J. (1995). An ethylene-inducible component of signal transduction encoded by *Never-ripe*. *Science* 270, 1807–1809.

Yamada-Okabe, T., Shimmi, O., Doi, R., Mizumoto, K., Arisawa, M. & Yamada-Okabe, H. (1996). Isolation of the mRNA-capping enzyme and ferric-reductase-related genes from Candida albicans. Microbiology 142, 2515-2523.

Received 19 August 1997; revised 14 October 1997; accepted 20 October 1997.